Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8801–8803, August 1999

Commentary

Slime molds, ascidians, and the utility of evolutionary theory

Leo W. Buss

Departments of Ecology & Evolutionary Biology and Geology & Geophysics, Yale University, New Haven, CT 06520-8106

Imagine playing this little variant of poker. You or any number of your colleagues can play. The game comes equipped with two decks of cards: one with 32 aces, 16 kings, and four queens and the other a conventional deck. The rules are these. Some external agency grants you funds sufficient to play a few hands. A machine deals the cards, choosing one or the other deck from which you will be dealt all hands. The cards are dealt in accord with an algorithm that reads a code emblazoned on the back of each card in infrared dye. When a card is dealt, the machine plays an annoying little tune of a few bars in length. Whoever gets a full house or better before his funds disappear wins a renewal of funds sufficient to play a few more hands.

This game is about discovering generality in biology. How so? Let's give the face values on the cards biological interpretations. An ace represents genes and molecules underlying biosynthetic pathways of great antiquity, the kings mechanisms of transcription and translation, the queens regulatory or signaling molecules. The cards comprising the stacked deck correspond to events that arose early in the history of life. The conventional deck, with its deuces and treys, represent taxon-specific biological features, say, the morphologies of antelope horns, the variety of social insect castes, or the polyp polymorphisms of siphonophores. A winning hand is a general finding, one germane to all or a large fraction of extant organisms.

There are two routes to biological generality, just as there are two ways to win our game. You can win the game either by playing with the stacked deck or by cracking the code that maps the tune to the draw. If the machine picks the stacked deck, you can hardly avoid getting a full house or better. Winning is largely a matter of getting into the game. Study of biological mechanisms that arose early in the history of life is the equivalent to playing our game with the stacked deck. The earlier the mechanism evolved, the more substantial the fraction of the extant forms of life that may be expected to display that mechanism or variants on its theme. Playing with the stacked deck yields many winners; generality is routinely achieved and achieved as an incidental byproduct of early origin and common descent.

The alternative strategy, that of cracking the code, works with either deck, but is obligatory if a player using the conventional deck is to win. Genealogy provides no built-in route to generality for those biologists enthralled with late-evolving features idiosyncratic to specific taxa. Generality can be realized with the deck stacked against you, but it requires a hypothesis of the process governing the distribution of the features, just as in our game we must hypothesize some map between the cards dealt and the tune we hearing playing in our heads.

Unlike the players in our game, real researchers have the option of choosing the deck with which to play. Yet they must respect the rule that one's ante is renewed only with a full house or better and they play in a world where the house has become dependent on its cut of every ante. We cannot but expect that the houses will be disproportionately stocked by, and that the winning hands filling our journals largely emanate from, those players who chose the deck where generality comes, so to speak, for free. It is appropriate, though, that we celebrate, with however modest a commentary, those occasional instances when a process is inferred that permits us to assemble a winning hand from the fair deck. The value of such stories does not lie solely in the smile they bring to those scholars whose tastes run to code breaking in the face of dangerously few chips. Rather, such stories serve as a useful reminder that the universe of symbols soon will be largely characterized and, soon enough, we shall all become code breakers once again.

Ascidian Allorecognition

Grab an algal frond at low tide from many a near-shore environment and you may well find it covered by colonies of the botrylloid ascidian genus *Botryllus*. These strikingly beautiful animals are chordates; although they have a decidedly invertebrate presentation as a surface encrustation, the larva is a proper tadpole, notochord and all. Your algal frond likely bears a number of such colonies, the margins of which abut one another. Events that occur at such margins have attracted the attention of Irv Weissman's group, whose report in this issue of the *Proceedings* (1) will serve as the first card from which we will attempt to assemble a winning hand.

When two *Botryllus* colonies grow into contact, one of two results are obtained. Either the colonies fuse, vascular continuity is established and genetic chimera results, or the colonies reject with each colony retaining its status as a distinct physiological and genetic individual. Fusion and rejection, as Oka and Wantanabe (2) suggested and Weissman's group (3) confirmed years ago, are alternatives encoded as a single, codominant Mendelian trait. Colonies bearing one or both alleles at the Fu/HC locus fuse, those sharing no alleles reject. Natural populations support multiple alleles at this locus (4), so colonies that fuse are likely to be kin.

On the face of it, the choice to fuse or reject would appear to be a choice between competing and cooperating. When two colonies reject, those colonies compete in the conventional sense an ecologist would recognize and label as interference competition, that is, one colony physically prevents access of the other to a resource, space on the algal frond, that is locally limiting. Stoner *et al.* (1) show us that the fusion event, the superficially cooperative behavior, in fact involves a competition at well.

Botryllus, like all clonal invertebrates, does not sequester a germ line. When colonies fuse, there is the potential that stem cells of one colony may be exchanged with those of the fusion partner. This is an evolutionarily very dangerous game to play; what if the stem cells from one fusion partner were to prove capable of becoming disproportionately represented in the gametes? If so, one colony has effectively become parasitized by the other. Stoner *et al.* (1) show that this is precisely what occurs. The authors identified a set of microsatellite markers diagnostic for particular colonies, established fusions between size-standardized colonies, and then assayed for the presence

The companion to this Commentary begins on page 9148.

or absence of markers in somatic tissues and for the frequency of the markers in sperm. The findings show clearly that the consequence of fusion is the disproportionate representation of fusion partners in both the somatic and gametic compartments. Some colonies are clear germ-line winners: they are disproportionately represented in the gametes. Indeed, in fully one-third of all pairwise combinations tested, one colony had pimped the other out of its germ line. The choice between competing and cooperating is an illusion, rather the choice is one of competing at the level of the individual or competing at the level of the cell lineage.

Stoner *et al.* (1) further show that germ-line winners need not achieve disproportionate somatic representation to outcompete fusion partners for access to the gametes, that their more extensive analysis of sperm holds true for their more limited assays of eggs, that the results assayed at one time point are similar to those generated for chimeras permitted to remain fused for a more extended duration, that the results are not only repeatable across replicates, but also obtain when the chimera involves three components, and that each colony can be placed into a rough hierarchy of somatic and gametic competitive ability. The latter result, that a superior germ-line competitor is not necessarily a somatic cell "winner" certainly suggests that the germ-line competitors may actively direct fusion partners to somatic tasks.

The colonies used in this analysis were drawn from a known pedigree. Stoner *et al.* (1) show that the propensity for germ-line parasitism is nonrandomly distributed in the pedigree and from this they infer the trait to be heritable. The latter will elicit some complaints, for they have not demonstrated that the frequency of the trait has increased with selection for the trait, nor have they provided an estimate of heritability by using the conventional narrow sense mating designs and analysis of covariance (5). Heritability, with the word taken to refer to a statistic as opposed to the capacity to be inherited, is a matter of partitioning variance under an assumption of additive genetic effects. The quantitative measure is the stock in trade of those who have no intent of isolating and characterizing genes, whereas pedigrees are a prominent tool of those who find genes, at least those who do so by positional cloning techniques. The rapid progress of Weissman's group (1) in identifying and mapping the chromosomal interval spanning the Fu/HC locus by using bulk segregant analysis (6) is germane here. For many, however, the heritability claim will remain a claim until either the conventional analysis is performed or loci contributing to successful germ-line competitive ability are localized.

The findings of Stoner *et al.* (1) confirm a prediction made in this journal some time ago (7) that allorecognition phenomena in clonal invertebrates and analogous phenomenon in some ascomyctes and myxomycetes serve to prevent germ-line parasitism. Under this hypothesis, the benefits of fusion, which include size increase and chimeric vigor (7), are offset by the potential costs of germ-line parasitism. Because complete germ-line parasitism is the evolutionary equivalent of death, fusion is dangerous and must be prevented, or restricted to close kin, by devices like allorecognition. The original perspective was a code-breaking sort of suggestion; a process inferred from the observation that taxa that display allorecognition phenomena of this sort are disproportionately taxa that do not sequester their germ lines and, hence, are susceptible to germ-line parasitism. We count Stoner *et al.*'s findings (1) as a card for our hand.

Slime Mold Cheaters

Eighteen years ago, a *Dictyostelium* strain was collected by a guy lying on a pile of horse dung behind a barn in Hamden, CT. Oddly enough, it was work on this slime mold isolate that spawned the original suggestion linking invertebrate allorecognition to competition for access to the germ line (7). Recall that cellular slime molds are organisms whose curious life cycle is played out in the soil. Spores germinate to produce free-living amoebae that proliferate on a diet of bacteria. When bacterial populations are exhausted, the free-living cells aggregate to form a multicellular grex that eventually develops into a fruiting structure composed of (somatic) stalk and (germinative) spores. The horse dung strain was interesting in that it produced spores, but no stalk. In coaggregates with wild types, it contributed nothing to the somatic duties of producing the stalk, the strain behaved as a germ-line parasite (7).

Herbert Ennis, Richard Kessin, and colleagues deal us our second card. In a recently submitted manuscript, they present results that I here briefly summarize as a personal communication. Using an insertional mutagenesis scheme now available for slime molds, they have recovered a strain that resembles the horse dung strain. They call it a cheater. The selection scheme used to identify cheaters was a clever one favoring strains that behaved as germ-line parasites. Mutagenized cells were passed through some 20 asexual generations, after which individual clones were characterized. The idea here is that an amoeba with an insertion in a gene that generated a parasitic phenotype would increase in frequency with each generation.

The selection scheme was successful in identifying a cheater strain. The cheater A, *chtA,* phenotype is fascinating. When cultured in isolation, the strain produces proper grexes, but the grexes fail to form a fruiting stage. However, when *chtA* cells are allowed to coaggregate with wild types, *chtA* cells produce spores, but fail to contribute to the somatic compartment. Moreover, the effect is likely more than a simple passive failure to form stalk. Rather the rate of increase of the *chtA* in chimeras is such that the *chtA* either suppresses formation of spores or causes the wild type to generate stalk. From the perspective of the *chtA* strain, coaggregation delivers chimeric vigor, the wild type compensates for the *chtA*'s inability to produce stalk. From the perspective of the wild type, coaggregation is infection by an obligate germ-line parasite.

Cheater A is a null mutant. In wild-type strains, the gene is expressed in grex stages, when the stalk and spore prepatterns are established, but not in the amoeboid phase of the life cycle, when cells display no hint of differentiation. The mutated gene encodes a protein bearing a F-box and WD40 repeats, leading Ennis *et al.* to suggest that *chtA* acts to remove, or to regulate the removal, of a protein required in the transition from grex stage to the fruiting body stage, presumably targeting it for ubiquitination and subsequent degradation. This explanation is attractive in that it accounts for one aspect of the observed phenotype, the inability of *chtA* cells to generate fruiting structures. The other aspect of the phenotype, the *chtA*'s suppression of spore formation and/or induction of stalk formation, is no less intriguing. Ennis *et al.* provide a plausible model. If the protein targeted for degradation by *chtA* regulates the secretion of products that specify cell fate (e.g., differentiation-inducing factor; ref. 8), both aspects of the *chtA* phenotype are accommodated. Note, as Ennis *et al.* do, that mutants of the hypothesized *chtA* target should rescue *chtA* cells, allowing them to produce spores, thus providing a clear avenue to identification of the *chtA* targets using the same insertional mutatgenesis strategy that led to identification of the *chtA* itself.

Just as in the case of the botrylloid ascidians, selection can act at the level of the multicellular individual or at the level of the cell lineage. The stalk of the cellular slime mold serves to project the spores into the interstices of the soil where an isopod or the like may well be traveling. The isopod selects for multicellularity. Selection at the level of the multicellular individual does not alone imply cooperation within. The life cycle of the slime mold provides no guarantee of genetic homogeneity of the coaggregate. Some cells must become stalk, but to do so is evolutionary death. Coaggregation with

cheaters like *chtA* poses the same dilemma for a slime mold as does indiscriminate fusion for an ascidian. We have now a hand with two of a kind.

Major Transitions in the History of Life

Ennis *et al.*'s work on slime mold cheaters and Stoner *et al.*'s (1) findings on ascidian allorecognition bear commonality in process. Although it is conceivable that the *chtA* locus and its targets someday will be shown to be homologous to the molecules that govern allorecognition and subsequent germ-line parasites in ascidians, there is no necessary reason for the systems to involve homologous elements. Code breaking can yield commonality with or without descent. The two systems may well be two of a kind only in terms of evolutionary context and process.

Two deuces, however, are still a long way from a full house. The hand is filled out by the recognition that the evolutionary context exemplified in these two studies defines a context that must have arisen, and been resolved, at each of the major transitions in the history of life.

Consider a social insect colony. The colony as a unit reproduces. The ants within a colony reproduce. The cells within each ant reproduce. The mitochondria with each cell reproduce, as does the chromosome of the mitochondria and those of the nucleus. And within those chromosomes are likely transposable elements bearing sequences for gene-processing enzymes allowing them to reproduce as autonomous units. Any modern organism is a Russian doll of actually or potentially reproducing units. Selection can, in principle, act on each such unit. We will, of course, not see its operation in most modern contexts, because conflict between units of selection are evident only when a chimera is formed.

Chimeras that pit one unit of selection against another arise in specific ecological contexts, as exemplified by Ennis' slime molds or Weissman's ascidians. They also can arise by mutation during the life of an individual, as any oncologist knows well. But far more importantly, they must necessarily have arisen at specific intervals in the history of life. To generate the Russian doll motif, one actually reproducing unit must have became enclosed within or become an elaborated part of another. At each of the major transitions in evolution, when one reproducing, selectable unit became a part of another, chimeras are inevitable. And just as allorecognition likely evolved to control the germ-line parasitism and the suppressor mutants being sought by Ennis *et al.* likely reflect responses to his *chtA* strains, each such conflict in the history of life generated a conflict ultimately resolved by subsequent adaptations.

The recognition that the history of life is a history of conflicts between units of selection and that higher-level units persist only when conflicts at the lower level are suitably constrained by subsequent adaptation was first elaborated more than a decade ago (9). The proposal since has been vastly extended and popularized (10), and book-length treatments include refs. 9–13. Substantive analyses are now available treating, for example, for the origin of the chromosome, the origin of the eukaryotic cell, the origin of multicellularity and cellular differentiation, and the origin of coloniality. Each such analysis yields predictions as to how features characteristic of particular levels of biological organization serve as adaptations to restrict the spread of parasites acting at the lower level. A full house or better!

Biology is not, as I recall once reading in a commentary much like this one, the "science of the arbitrarily idiosyncratic." What at first appears to be perversely complex and arbitrarily idiosyncratic is never so when placed in genealogical context. What at first seems arbitrary becomes history. Even more striking, at least to me, are cases like those touched on here, where genealogically unrelated phenomena are seen as responses to a common process. These cases are not merely history; they address why we have the history we have. They permit us a glimpse of the ghost in that machine that plays the tune and deals us our cards.

Homayoun Bagheri, Luis Cadavid, and Jim Griesemer kindly provided comments on these comments. I thank Herb Ennis and Rich Kessin for permission to discuss their forthcoming manuscript.

1. Stoner, D. S., Rinkevich, B. & Weissman, I. L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 9148–9153.
2. Oka, H. & Wantanabe, H. (1957) *Proc. Jpn Acad. Sci.* **33,** 657–659.
3. Scofield, V. L., Schlumpberger, J., West, L. A. & Weissman, I. L. (1982) *Nature (London)* **295,** 499–502.
4. Tanaka, K. & Wantanabe, H. (1973) *Cell. Immunol.* **7,** 410–426.
5. Falconer, D. S. & MacKay, T. F. C. (1996) *Introduction to Quantitative Genetics* (Addison–Wesley Longman, Essex, U.K.).
6. De Tomaso, A. W., Saito, Y., Ishizuka, K. J., Palmeri, K. J. & Weissman, I. L. (1998) *Genetics* **149,** 277–287.
7. Buss, L. W. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5337–5341.
8. Kay, R. R. (1998) *J. Biol. Chem.* **273,** 2669–2675.
9. Buss, L. W. (1987) *The Evolution of Individuality* (Princeton Univ. Press, Princeton).
10. Maynard Smith, J. & Szathmary, E. (1999) *The Origins of Life: From the Birth of Life to the Origin of Language* (Oxford Univ. Press, Oxford).
11. Maynard Smith, J. & Szathmary, E. (1995) *The Major Transitions in Evolution* (Freeman, San Francisco).
12. Wilson, D. S., ed. (1997) *Am. Nat.* **150,** S1–S134.
13. Michod, R. E. (1999) *Darwinian Dynamics: Evolutionary Transitions in Fitness and Individuality* (Princeton Univ. Press, Princeton).